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REMARKS

The specification has been amended above to include a cross-reference of the parent applications. No new matter has been added.

The applicants elect the subject matter the Examiner's Group III for further prosecution of the above. With regard to the species election requirements the applicants elect the structure of SEQ ID NO:1 recited in item (b) of claim 24. With regard to the Examiner's comment relating to substitutions of the amino acids, the applicants elect SEQ ID NO:1 *per se* having no substitutions. As for the polyalkylene derivative which modifies the polypeptide, the applicants elect the polyalkylene derivative having a structure of the formula (I) recited in item (a) of claim 29.

The specification has been amended above to include the attached paper copy of the Sequence Listing which is the same as the paper and computer readable copies of the Sequence Listing filed in the parent application Serial No. 08/696,988. The Office is requested to use the computer readable copy of the Sequence Listing filed in the parent application Serial No. 08/696,988, for the present application. A separate Request to this effect is attached. No new matter has been added.

Also attached are copies of Declarations, both executed and unexecuted, by Motoo Yamasaki, filed in the parent application Serial No. 08/696,988, for completeness. The executed Declarations are dated May 26, 1999, and August 28, 1998.

Also attached are copies of Tables 1 and 2 (two pages), which were submitted with the Amendment of February 4, 2000, in the parent application Serial No. 08/696,988.

YAMASAKI et al
Appl. No. 10/084,615
May 20, 2004

A copy of the Declaration relating to the deposit of biological materials filed in the parent application Serial No. 08/696,988, along with a copy of the deposit receipt are also attached.

The Examiner in the parent application Serial No. 08/696,988, confirmed receipt of the certified copy of the priority document in, for example, the Notice of Allowability, dated October 25, 2000. The present Examiner is requested to confirm the same in this application.

Attached is a Request for a Corrected Filing Receipt which will properly indicate that the parent PCT application is a domestic priority claim, as the parent application is a 371 U.S. National Phase of a PCT application, as opposed to being a foreign priority claim. Return of a Corrected Filing Receipt is requested.

Return of an initialed copies of the three (3) PTO-1449 Forms previously filed, pursuant to MPEP § 609, is requested in the Examiner's next communication. Moreover, return of the attached PTO-1449 Form, which lists Abstracts cited by the Examiner in the parent application in an Office Action dated November 23, 1998, copies of which are attached hereto, is requested, pursuant to MPEP § 609.

The claims have been amended to rewrite claim 24 as an independent claim.

The applicants note that, of the elected claims, claims 24 and 28-33 read on both elected species.

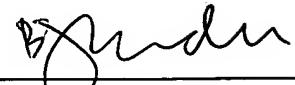
An early and favorable Action on the merits of the claimed invention is requested.

YAMASAKI et al
Appl. No. 10/084,615
May 20, 2004

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____



B. J. Sadoff

Reg. No. 36,663

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Facsimile: (703) 816-4100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re Patent Application of

YAMASAKI et al

Atty Ref. 249-88

Serial No. 08/696,988

Group:

Filed: August 16, 1996

Examiner:

For: PLATELET PRODUCTION
PROMOTING AGENT

* * * * *

Honorable Commissioner of Patents and
Trademarks
Washington, DC 20231

**DECLARATION RE: DEPOSITED
BIOLOGICAL MATERIALS**

Sir:

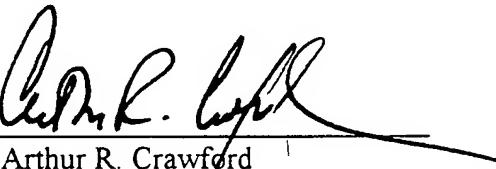
As an authorized representative and on the instructions of **Kyowa Hakko Kogyo Co., Ltd.** of Tokyo, Japan, the assignee of the U.S. patent application identified above, I hereby declare that:

- **Kyowa Hakko Kogyo Co., Ltd.** is also the depositor and owner of the *Escherichia coli* ECfBD28 accession number FERM BP-1479 deposited September 18, 1987, identified and referred to in the specification at page 32 of this application and on the attached deposit receipt, international form, deposited under the terms of the Budapest Treaty.
- The deposit of biological material identified above was made at the Fermentation Research Institute of the Agency of Industrial Science and Technology, 1-3, Higashi 1-chome, Yatabe-machi Tsukuba-shi, Ibaraki 305 Japan and was deposited and accepted under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and therefore the filing of a viability statement is unnecessary (37 CFR 1.807(b)).

- This deposit will be maintained for a period of 30 years from the date of deposit or for the enforceable life of any patent issuing from this application or for a period of 5 years after the date of the most recent request for the furnishing of a sample of the deposited material, whichever is longest.
- The deposit will be replaced should it become contaminated or no longer viable.
- Subject to 37 CFR 1.808(b), all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent.
- Access to the deposited material is permitted during the pendency of the above-identified patent application to one determined by the Commissioner of Patents and Trademarks to be so entitled under 37 CFR §1.14 and 35 USC §122.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Nov. 6, 1991
Date

By: 
Name: Arthur R. Crawford
Reg. No. 25,327



PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Motoo Yamasaki et al.

Appln. No.: 08/696,988 Group Art Unit: 1654

Filed: August 16, 1998 Examiner: Delaney, R

For: PLATELET PRODUCTION PROMOTING AGENT

DECLARATION UNDER 37 C.F.R. §1.132

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir/Madam:

I, Motoo Yamasaki, do declare and state that:

I graduated from Tokyo Instituted of Technology in March, 1978, finished postgraduated Master course of Tokyo Institute of Technology in March, 1980 and have been employed since April, 1980 by Kyowa Hakko Kogyo Co., Ltd., the assignee of the above-identified application. I worked at Tokyo Research Laboratories of the company in July, 1980.

I studied at National Cardiovascular Center Research Institute from April, 1990 to July, 1991.

I am a co-inventor of the above-identified patent application and am familiar with the Office Action dated February 24, 1998 in the above-identified application.

In order to demonstrate the superiority of the present invention, the following comparative

U.S. APPLICATION NO. 08/696,988
DECLARATION UNDER 37 C.F.R. §1.132

PATENT APPLICATION

experimentation was conducted by me or under my direct supervision.

EXPERIMENTATION

Influences of hG-CSF and chemically modified hG-CSF on the recovery of reduced platelets in total-body irradiating mice

1. Experimentation method

Experimentation was carried out according to Experimental example 2 in the present specification as follows.

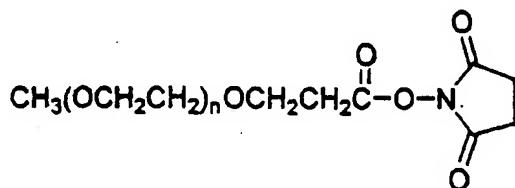
Total-body irradiation (3Gy/mouse) was carried out for 5 male BALB/c mice (10 weeks of age) by using an X-ray irradiation apparatus (MBR-1520, Hitachi Medical Corporation, Tokyo).

The day after irradiation, intact-hG-CSF or chemically modified hG-CSF prepared by using the following chemical modifier (average molecular weight: about 20,000) according to Reference example 19 in the present specification was administered subcutaneously at a single dose of 0 to 1 μ g per mouse. PEG-modified G-CSF which was considered by the Examiner to be equivalent to

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SM-modified G-CSF was used as the chemically modified G-CSF. Water and feed were available ad libitum.



Blood was sequentially collected from the murine vein of eyeground, and the peripheral platelet count was determined using an automatic cell counter (F-820, TOA MEDICAL ELECTRONICS CO., LTD., Kobe).

2. Results

The results are shown in Fig. 1 as attached herewith. The results of mice to which hG-CSF was administered are shown by —x— and —+—. The results of mice to which the chemically modified hG-CSF was administered are shown by —■— and —▲—. The results of mice untreated was shown by —◆—.

In the mice to which hG-CSF or the chemically modified hG-CSF was not administered, the peripheral platelet count was apparently decreased, and became the

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minimum on the ninth day. After then, it was gradually recovered.

In the mice to which the chemically modified hG-CSF was administered, the peripheral platelet count was apparently recovered. On the other hand, in the mice to which hG-CSF was administered, such recovering effect could not be recognized.

The results show that, although G-CSF has no platelet production promoting effect, chemically modified G-CSF has platelet production promoting effect. That is, the present invention can provide unexpected results.

**U.S. APPLICATION NO. 08/696,988
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PATENT APPLICATION

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date : _____ Name : _____

Motoo Yamasaki

**PATENT APPLICATION****IN THE UNITED STATES PATENT AND TRADEMARK OFFICE****In re application of****Motoo Yamasaki et al.****Appln. No.: 08/696,988****Group Art Unit: 1654****Filed: August 16, 1998****Examiner: Delaney, R****For: PLATELET PRODUCTION PROMOTING AGENT****SECOND DECLARATION UNDER 37 C.F.R. §1.132****Assistant Commissioner for Patents
Washington, D.C. 20231****Sir/Madam:****I, Motoo Yamasaki, do declare and state that:****I am the same Motoo Yamasaki, who executed in the
previous Declaration on August 28, 1998.****In order to demonstrate the superiority of the
present invention, the following comparative
experimentation was conducted by me or under my direct
supervision.****EXPERIMENTATION****1. Preparation of SM-modified hG-CSF****To 2 mL of a human granulocyte colony stimulating
factor (referred to as hG-CSF) solution which had been
adjusted to 4 mg/mL with a 0.5 M aqueous sodium
bicarbonate solution containing 0.1% Tween 80, 0.5 mL of
a dimethylsulfoxide solution containing 8.1 mg of
poly(styrene-maleic anhydride) (SM) (SMA2625 type;**

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PATENT APPLICATION

average molecular weight: 1,900) was added and allowed to react at 4°C for 24 hours. The resultant mixture was centrifuged at 15,000 rpm for 20 minutes, and the supernatant was purified by an ion-exchange column using Q-Sepharose Fast Flow resin (Amersham-Pharmacia Biotech, resin amount: 1.5 mL). The fraction containing no unmodified hG-SCF and unreacted SM was collected to obtain 3 mL of a 1.75 mg/mL SM-hG-CSF solution (yield: 66%).

2. Preparation of SM-modified hG-CSF derivative

To 2 mL of an hG-SCF derivative (prepared in Reference Example 3 of the present specification) solution which had been adjusted to 4 mg/mL with a 0.5 M aqueous sodium bicarbonate solution containing 0.1% Tween 80, 0.5 mL of a dimethylsulfoxide solution containing 8.1 mg of poly(styrene-maleic anhydride) (SM) (SMA2625 type; average molecular weight: 1,900) was added and allowed to react at 4°C for 24 hours. The resultant mixture was centrifuged at 15,000 rpm for 20 minutes, and the supernatant was purified by an ion-exchange column using Q-Sepharose Fast Flow resin (Amersham-Pharmacia Biotech,

U.S. APPLICATION NO. 08/696,988
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PATENT APPLICATION

resin amount: 1.5 mL). The fraction containing no unmodified hG-SCF derivative and unreacted SM was collected to obtain 3 mL of a 1.26 mg/mL SM-hG-CSF solution (yield: 47%).

3. Platelet Production promoting effect

X-ray irradiation of 300 roentgens was carried out for male BALB/c mice (7 weeks of age, purchased from Charles River Japan Inc.) by using an X-ray irradiation apparatus (MBR-1520, produced by Hitachi Medical Corporation). Then, the above SM-hG-CSF or SM-hG-CSF derivative was diluted with physiological saline, and was injected subcutaneously into the X-ray irradiated mouse. A few days after the injection, blood was sequentially collected from the murine vein of eyeground, and the platelet count was determined using an automatic cell counter. The platelet count of mouse was determined prior to the X-ray injection as a control. The change of the platelet count was calculated as a ratio (%) to the control. Also, the experiment was carried out using 5 mice per group, and an average was used.

The results are shown in Table 1 below.

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 DECLARATION UNDER 37 C.F.R. §1.132

PATENT APPLICATION

TABLE 1
 Platelet production promotion effect
 in X-ray irradiated mouse

Sample	Dose (μg)	Day after the irradiation (Days)	Mean platelet count (%)
Untreated	-	11	36.8
SM-hG-CSF	1	11	45.6
SM-hG-CSF derivative	0.2	11	39.9
Untreated	-	7	40.9
SM-hG-CSF	1	7	48.2
SM-hG-CSF derivative	0.2	7	47.8

Platelet production promotion effect was confirmed in both the SM-modified hG-CSF and the SM-modified hG-CSF derivative.

U.S. APPLICATION NO. 08/696,988
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PATENT APPLICATION

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date : May 26, 1999 Name : Motoo Yamasaki

Motoo Yamasaki



PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Motoo Yamasaki et al.

Appln. No.: 08/696,988

Group Art Unit: 1654

Filed: August 16, 1998

Examiner: Delaney, R

For: PLATELET PRODUCTION PROMOTING AGENT

SECOND DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir/Madam:

I, Motoo Yamasaki, do declare and state that:

I am the same Motoo Yamasaki, who executed in the previous Declaration on August 28, 1998.

In order to demonstrate the superiority of the present invention, the following comparative experimentation was conducted by me or under my direct supervision.

EXPERIMENTATION

1. Preparation of SM-modified hG-CSF

To 2 mL of a human granulocyte colony stimulating factor (referred to as hG-CSF) solution which had been adjusted to 4 mg/mL with a 0.5 M aqueous sodium bicarbonate solution containing 0.1% Tween 80, 0.5 mL of a dimethylsulfoxide solution containing 8.1 mg of poly(styrene-maleic anhydride) (SM) (SMA2625 type;

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PATENT APPLICATION

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U.S. APPLICATION NO. 08/696,988
DECLARATION UNDER 37 C.F.R. §1.132

PATENT APPLICATION

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U.S. APPLICATION NO. 08/696,988
 DECLARATION UNDER 37 C.F.R. §1.132

PATENT APPLICATION

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U.S. APPLICATION NO. 08/696,988
DECLARATION UNDER 37 C.F.R. §1.132

PATENT APPLICATION

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Date : _____ Name : _____

Motoo Yamasaki

〔特許手続上の微生物の国際的承認〕
に関するブダペスト条約

下記国際寄託当局によって規則 7.1に従い
発行される

原寄託についての受託証

寄託者 氏名（名称）協和醸酵工業株式会社
取締役社長 加藤幹夫
あて名 東京都千代田区大手町一丁目6番1号 展設

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITORY AUTHORITY identified at the bottom of this page.

I. 微生物の表示	
(寄託者が付した識別のための表示)	(受託番号)
Escherichia coli ECfBD28	微工研条寄第 1479号 (FERM BP-1479)
II. 科学的性質及び分類学上の位置	
I欄の微生物には、次の事項を記載した文書が添付されていた。 <input type="checkbox"/> 科学的性質 <input type="checkbox"/> 分類学上の位置	
III. 受領及び受託	
本国際寄託当局は、昭和62年 9月18日（原寄託日）に受領したI欄の微生物を受託する。	
IV. 国際寄託当局	
通商産業省工業技術院微生物工業技術研究所	
名称:	Fermentation Research Institute Agency of Industrial Science and Technology
所長	佐藤昭雄 Akio Sato, Dr., DIRECTOR GENERAL.
あて名: 日本国茨城県筑波郡谷田部町東1丁目1番3（郵便番号 305) 1-3, Higashi 1 chome Yatabe-machi Tsukuba-gun Ibaraki-ken 305, JAPAN	
昭和62年(1987) 9月18日	

TABLE 1

Strain	Plasmid	Product of Plasmid	Specific activity Unit/mg	Specific activity
ECfTA1	pCfTA1	G-CSF(intact)	2.8 x 10 ⁸	1.0
ECfTL38	pCfTL38	G-CSF(Ser ¹)	4.0 x 10 ⁸	1.8
ECfTL41	pCfTL41	G-CSF(Arg ¹)	3.7 x 10 ⁸	1.7
ECfTL23	pCfTL23	G-CSF(Gly ¹)	3.1 x 10 ⁸	1.4
ECfTL35	pCfTL35	G-CSF(Cys ¹)	2.9 x 10 ⁸	1.3
ECfBB101	PCfBB101	G-CSF(NB101)	7.9 x 10 ⁸	3.6
ECfBC42B1	pCfBC42B1	G-CSF(NC42B1)	5.1 x 10 ⁸	2.3
ECfBC45	pCfBC45	G-CSF(NC45)	7.0 x 10 ⁸	3.2
ECfBC52	pCfBC52	G-CSF(NC52)	6.2 x 10 ⁸	2.8
ECfBC59	pCfBC59	G-CSF(NC59)	5.9 x 10 ⁸	2.7
ECfBC76	PCfBC76	G-CSF(NC76)	6.2 x 10 ⁸	2.8
ECfBC77	pCfBC77	G-CSF(NC77)	7.7 x 10 ⁸	3.5
ECfBC93	pCfBC93	G-CSF(NC93)	9.2 x 10 ⁸	4.2
ECfBC95	pCfBC95	G-CSF(NC95)	9.5 x 10 ⁸	4.3
ECfBC97	pCfBC97	G-CSF(NC97)	8.6 x 10 ⁸	3.9
ECfBD28	PCfBD28	G-CSF(ND28)	7.9 x 10 ⁸	3.6
ECfBD56	pCfBD56	G-CSF(ND56)	5.1 x 10 ⁸	2.3
ECfBD82	pCfBD82	G-CSF(ND82)	4.6 x 10 ⁸	2.1
ECfTM14	pCfTM14	G-CSF(Ser ¹ , Cys ¹)	3.1 x 10 ⁸	1.4
ECfTM17	PCfTM17	G-CSF(Ser ¹ , Arg ¹)	3.7 x 10 ⁸	1.7
ECfTM113	pCfTM113	G-CSF(Ser ¹ , Ser ¹⁷)	2.9 x 10 ⁸	1.3
ECfTNS7	PCfTNS7	G-CSF(Δ1-4S)	9.8 x 10 ⁸	3.5
ECfTAArg4S	pCfTAArg4S	G-CSF(Arg ⁴ , Ser ¹⁷)	7.3 x 10 ⁸	2.6
ECfTNS301	PCfTNS301	G-CSF(Δ1-11S)	3.9 x 10 ⁸	1.4
ECfTNS401	pCfTNS401	G-CSF(Δ1-7S)	7.0 x 10 ⁸	2.5
ECfTNS501	pCfTNS501	G-CSF(Δ1-6S)	5.6 x 10 ⁸	2.0
ECfB028A17	pCfB028A17	G-CSF(ND28A17)	8.7 x 10 ⁸	3.1
ECfB028T17	PCfB028T17	G-CSF(ND28T17)	7.6 x 10 ⁸	2.7
ECfTN205	pCfTN205	G-CSF(Δ1-4)	5.3 x 10 ⁸	1.9

TABLE 2

R^{A1} PheLeuLeuLys R^{A2} LeuGluGlnValArgLysIleGlnGly
 AspGlyAlaAlaLeuGlnGluLysLeuCysAlaThrTyrLysLeuCys
 HisProGluGluLeuValLeuLeuGlyHisSerLeuGlyIleProTrp
 AlaProLeuSerSerCysProSerGlnAlaLeuGlnLeuAlaGlyCys
 LeuSerGlnLeuHisSerGlyLeuPheLeuTyrGlnGlyLeuLeuGln
 AlaLeuGluGlyIleSerProGluLeuGlyProThrLeuAspThrLeu
 GlnLeuAspValAlaAspPheAlaThrThrIleTrpGlnGlnMetGlu
 GluLeuGlyMetAlaProAlaLeuGlnProThrGlnGlyAlaMetPro
 AlaPheAlaSerAlaPheGlnArgArgAlaGlyGlyValLeuValAla
 SerHisLeuGlnSerPheLeuGluValSerTyrArgValLeuArgHis
 LeuAlaGlnPro

wherein R^{A1} represents amino acid residue, peptidyl group selected from the group of ThrProLeuGlyProAlaSerSerLeuProGlnSer, SerProLeuGlyProAlaSerSerLeuProGlnSer, ArgProLeuGlyProAlaSerSerLeuProGlnSer, GlyProLeuGlyProAlaSerSerLeuProGlnSer, CysProLeuGlyProAlaSerSerLeuProGlnSer, AlaProThrArgSerAlaSerSerLeuProGlnSer, ThrProGluLysSerAlaSerSerLeuProGlnSer, ValProIleArgSerAlaSerSerLeuProGlnSer, CysProIleArgSerAlaSerSerLeuProGlnSer, TyrProIleArgSerAlaSerSerLeuProGlnSer, ArgProThrArgSerAlaSerSerLeuProGlnSer, ThrProThrArgSerAlaSerSerLeuProGlnSer, AsnProGluArgSerAlaSerSerLeuProGlnSer, IleProThrArgSerAlaSerSerLeuProGlnSer, SerProThrArgSerAlaSerSerLeuProGlnSer, AlaProSerAsnSerAlaSerSerLeuProGlnSer, AlaProProAsnArgGlySerSerLeuProGlnSer, SerProCysGlyProAlaSerSerLeuProGlnSer, SerProArgGlyProAlaSerSerLeuProGlnSer, SerProSerGlyProAlaSerSerLeuProGlnSer, ThrProLeuArgProAlaSerSerLeuProGlnSer, ThrProLeuArgProAlaSerSerLeuProGlnSer, AlaProThrTyrArgAlaSerSerLeuProGlnSer, AlaProThrTyrArgAlaSerSerLeuProGlnSer, ProAlaSerSerLeuProGlnSer, Ser, SerLeuProGlnSer, SerSerLeuProGlnSer and ProAlaSerSerLeuProGlnSer or a peptidyl group which has Met in the N terminal of the peptidyl group, and R^{A2} represents amino acid residue selected from the group of Cys, Ser, Ala and Thr.



PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Motoo Yamasaki et al.

Appln. No.: 08/696,988

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Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir/Madam:

I, Motoo Yamasaki, do declare and state that:

I graduated from Tokyo Instituted of Technology in March, 1978, finished postgraduated Master course of Tokyo Institute of Technology in March, 1980 and have been employed since April, 1980 by Kyowa Hakko Kogyo Co., Ltd., the assignee of the above-identified application. I worked at Tokyo Research Laboratories of the company in July, 1980.

I studied at National Cardiovascular Center Research Institute from April, 1990 to July, 1991.

I am a co-inventor of the above-identified patent application and am familiar with the Office Action dated February 24, 1998 in the above-identified application.

In order to demonstrate the superiority of the present invention, the following comparative

experimentation was conducted by me or under my direct supervision.

EXPERIMENTATION

Influences of hG-CSF and chemically modified hG-CSF on the recovery of reduced platelets in total-body irradiating mice

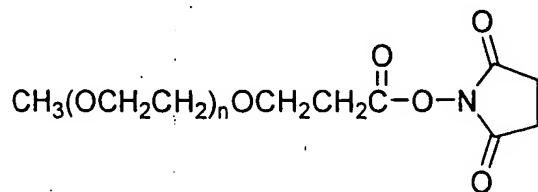
1. Experimentation method

Experimentation was carried out according to Experimental example 2 in the present specification as follows.

Total-body irradiation (3Gy/mouse) was carried out for 5 male BALB/c mice (10 weeks of age) by using an X-ray irradiation apparatus (MBR-1520, Hitachi Medical Corporation, Tokyo).

The day after irradiation, intact-hG-CSF or chemically modified hG-CSF prepared by using the following chemical modifier (average molecular weight: about 20,000) according to Reference example 19 in the present specification was administered subcutaneously at a single dose of 0 to 1 μ g per mouse. PEG-modified G-CSF which was considered by the Examiner to be equivalent to

SM-modified G-CSF was used as the chemically modified G-CSF. Water and feed were available ad libitum.



Blood was sequentially collected from the murine vein of eyeground, and the peripheral platelet count was determined using an automatic cell counter (F-820, TOA MEDICAL ELECTRONICS CO., LTD., Kobe).

2. Results

The results are shown in Fig. 1 as attached herewith. The results of mice to which hG-CSF was administered are shown by \times and $+$. The results of mice to which the chemically modified hG-CSF was administered are shown by \blacksquare and \blacktriangle . The results of mice untreated was shown by \blacklozenge .

In the mice to which hG-CSF or the chemically modified hG-CSF was not administered, the peripheral platelet count was apparently decreased, and became the

minimum on the ninth day. After then, it was gradually recovered.

In the mice to which the chemically modified hG-CSF was administered, the peripheral platelet count was apparently recovered. On the other hand, in the mice to which hG-CSF was administered, such recovering effect could not be recognized.

The results show that, although G-CSF has no platelet production promoting effect, chemically modified G-CSF has platelet production promoting effect. That is, the present invention can provide unexpected results.

U.S. APPLICATION NO. 08/696,988
DECLARATION UNDER 37 C.F.R. §1.132

PATENT APPLICATION

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date : August 28, 1998 Name : Motoo Yamasaki

Motoo Yamasaki